ON THE BEHAVIOUR OF THE THREAD-LIKE APPARATUS IN THE PETIOLE OF *MIMOSA PUDICA* L.

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§ 1. Introduction

The previous paper presented an integrated description of the thread-like apparatus based on knowledge obtained by means of fixing methods and vital staining[1]: The present author's study of the behaviour of the thread-like apparatus has hitherto been carried out by means of vital staining and, in some cases, by phase-contrast microscopy[2-8]. The present investigation was undertaken in view of the necessity to reconsider the problem of the behaviour of the thread-like apparatus in the living cell of the *Mimosa* petiole.

§ 2. Materials and methods

The material used in the present investigation was the petiole of *Mimosa pudica* L. The plants were grown in a field in pots exposed to the open air. Because the lacunal parenchymatous cells of petioles exhibit a well-developed thread-like apparatus connected with the tannin vacuole, these tissue-cells were selected for this study (Fig. 1). The lacunal parenchyma of the petiole was taken from adult plants, young and decrepit ones being avoided.

The sections of fresh material were prepared in the following manner. Petiole sections were cut 30–50 μ thick by the use of a hand microtome and a razor. Then the sections were immersed in a medium solution. All the sections were prepared fresh at the beginning of each observation.

§ 3. Detection of the thread-like apparatus in the living cell

In discussing the present experiments, we must discriminate carefully between the thread-like apparatus and protoplasmic threads. Furthermore, it is of fundamental importance to distinguish the thread-like apparatus from other cellular elements. We have attempted the cytochemical treatment of the intact cell on the basis of past experience[2]. The reaction of precipitation in the presence of caffeine occurred as one of the characteristics of the thread-like apparatus. The

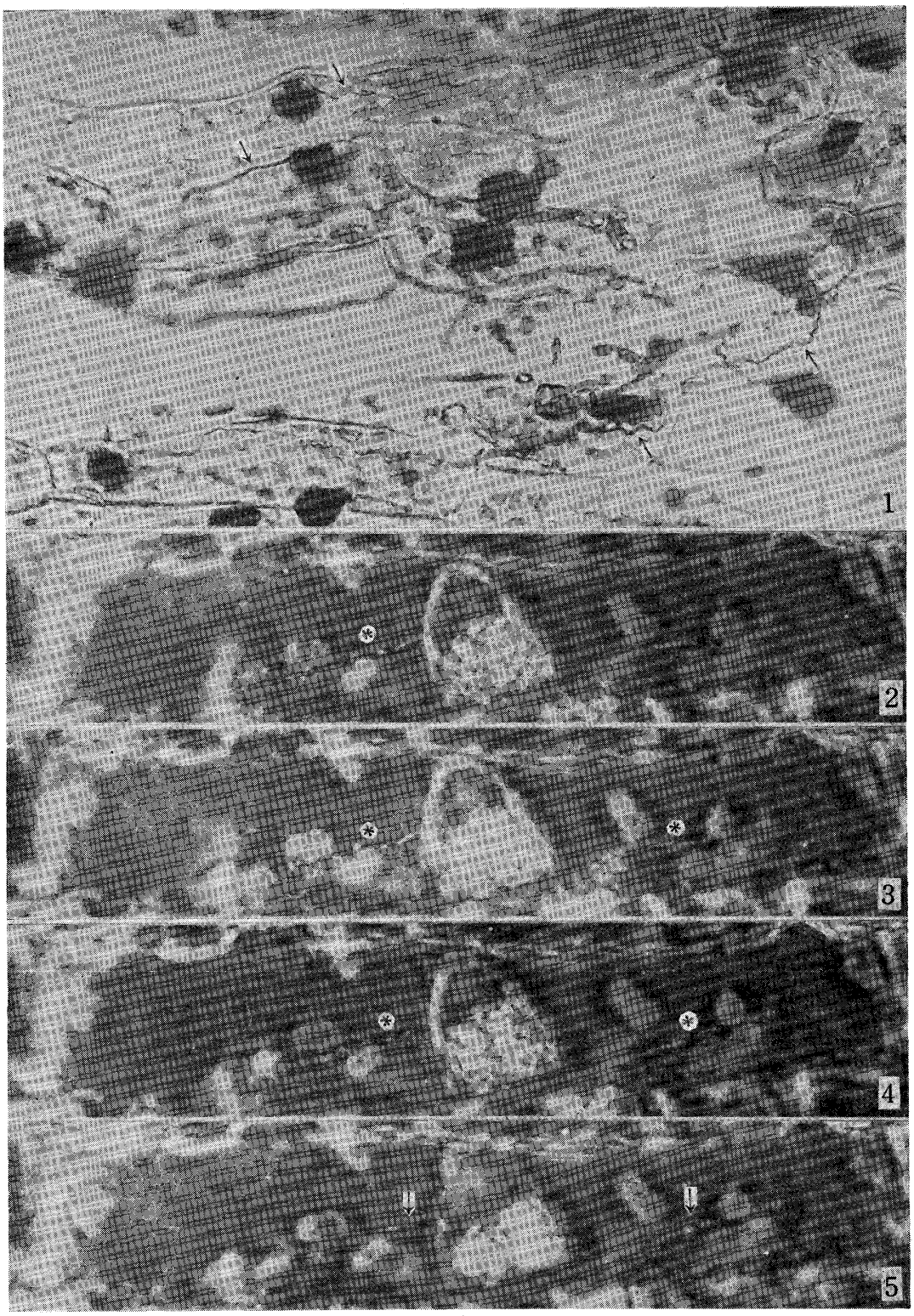


Fig. 1. Lacunal parenchyma of a *Mimosa* petiole cut longitudinally. Arrows indicate the thread-like apparatus. The material is fixed with Champy's fluid. (×700).

Figs. 2-5. Series of photographs of the precipitate with a caffeine solution. Figs. 2, 3, 4 in 0.1% caffeine; Fig. 5 in tap water. Arrows indicate the thread-like apparatus. Asterisks indicate the bead-like precipitation. (×1,150).

observations were made by phase-contrast microscopy in conjunction with a dark-medium-contrast 100 × objective with a "Periplan" 10 × ocular.

In one of the lacunal parenchymatous cells the tannin vacuole is located centrally and is connected on both sides with a thread-like apparatus which is attached to the chloroplasts. When a caffeine solution is used as the medium, a precipitation appears in the tannin vacuole. Furthermore, some parts of the thread show a bead-like precipitation, indicated by asterisks (Figs. 2, 3 and 4). These precipitations are capable of being transformed. Generally speaking, the caffeine solution produces precipitates positively in both the thread-like apparatus and tannin vacuoles.

Next, the medium was changed from caffeine to tap water. This procedure must be carried out swiftly and carefully. In the medium of tap water the thread-like apparatus assumes a linear shape in the same cell (Fig. 5) and begins to show a vermicular movement.

§ 4. Vermiculation of the thread-like apparatus

The sections from fresh materials were stained with 0.002 per cent chrysoidin. The staining was almost completed within 25 minutes. Figures 6a–g are a series of pictures of an intact cell which were taken at ten-minute intervals. During the course of this experiment, the threads and the tannin vacuoles varied in shape. The threads are capable of being transformed, becoming thicker when shortened or becoming thinner when stretching out. This process was also roughly followed by vital staining with neutral red and other dyes[3].

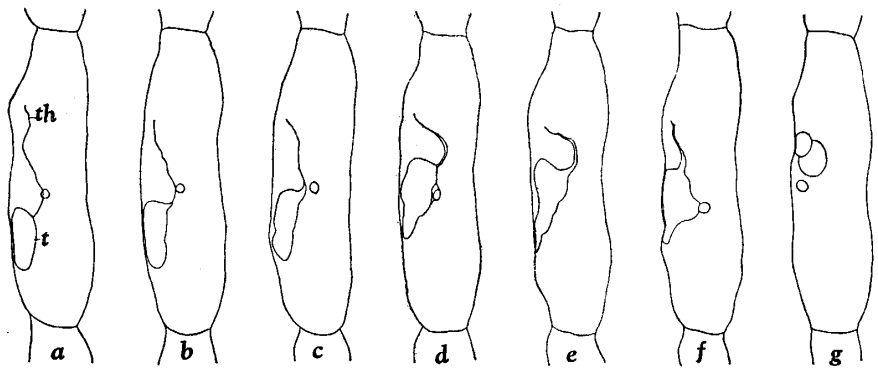


Fig. 6. Succesive shape of a cell observed at ten-minute intervals. th: thread-like apparatus; t; tannin vacuole (×400).

For the purpose of gaining a more complete knowledge of the behaviour of the thread-like apparatus, the author made detailed observations of the thread and other cellular elements. The observations were made by means of phase-contrast microscopy. The material was

soaked in tap water. As is shown in the series of photomicrographs (Figs. 7–10), the vermiculation of a part of the thread-like apparatus is observable. This vermiculation occurred continuously.

The author next made a series of observations using photomicrographs as in the foregoing experiment in order to determine the behaviour of the thread-like apparatus near the chloroplasts. With the material mounted in tap water, movements of the thread in the vicinity of some chloroplasts are observed in the intact cell (Figs. 11–14). The vermiculation of the thread-like apparatus near the chloroplasts is also observable. Sometimes the threads are transformed from the slender form into a bead-like shape near chloroplasts (Figs. 15–19). These facts suggeste that the thread-like apparatus is mounted in the protoplasm, which then moves with a sluggish protoplasmic streaming which is probably the chief motive force of the thread-like apparatus.

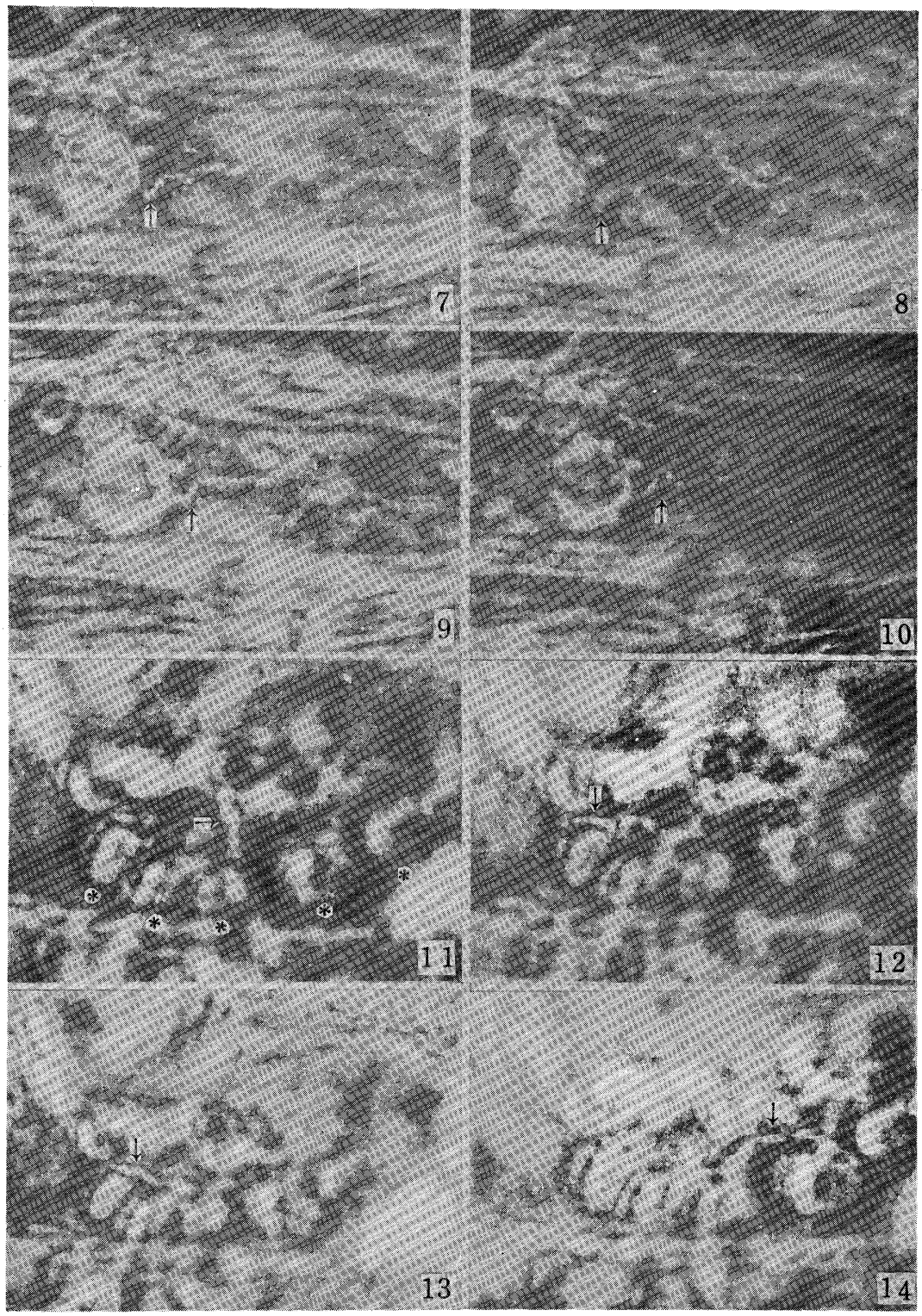
§ 5. Relation between the thread-like apparatus and chloroplasts

In the first experiment on this subject, the effect of light upon the thread-like apparatus and the chloroplasts was studied by means of vital staining. The longitudinal sections were stained with brilliant cresyl blue.

In the fresh material the tannin vacuoles in the lacunal parenchymatous cell, are more inflated under diurnal conditions, than under nocturnal conditions, and the thread-like apparatus in the daytime is thicker than in the nighttime. Furthermore, under nocturnal conditions the chloroplasts have a tendency to form a cluster around the thread-like apparatus. These differences are shown clearly in Figs. 19 and 20, in which both tissues are stained vitally with dye solutions. In the diurnal period the granules in chloroplast are stained more markedly by 0.001 per cent rhodamine B than those treated under nocturnal conditions (Figs. 21, 22). The threads and the granules in the chloroplasts are both stained within 30 minutes, and no distinct difference in the intensity of color is seen between these two cellular elements.

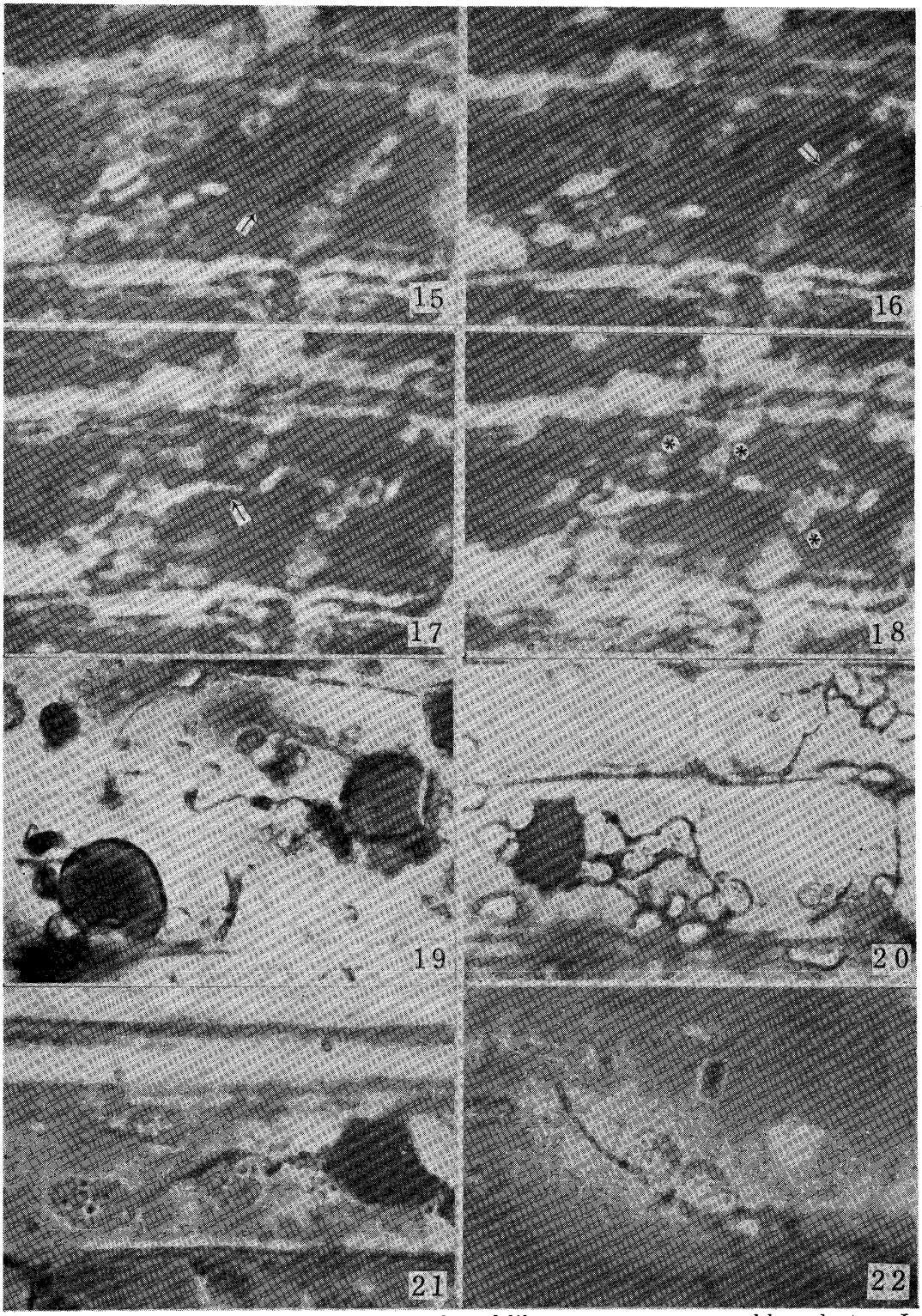
For the fixation of the lacunal parenchymatous cells, the author employed Champy's fluid. The sections were washed in running water and then in distilled water changed several times. The sections were mounted in pure glycerine, without any subsequent staining. This techniqe* not only gave excellent results in demonstrating the thread-like apparatus, but was also outstanding for the fixation of chloro-

*This method is called the Champy-Toriyama method in a previous paper (Toriyama, 1960a).



Figs. 7-10. Series of photographs of the vermiculation of the thread-like apparatus. The medium is tap water. Arrows indicate a part of the thread-like apparatus (×1600).

Figs. 11-14. Showing behaviour of the thread-like apparatus in vicinity of chloroplasts. The medium is tap water. Arrows indicate the thread-like apparatus. Asterisks indicate the chloroplast. (×1,600).



Figs. 15–18. Succesive changes in the thread-like apparatus near chloroplasts. In Figs. 15, 16, 17, arrows indicate the slender thread; Fig. 18, asterisks indicate the chloroplasts. The medium is tap water. (×1,150).

Figs. 19, 20. Longitudinal section of parenchyma. 19, diurnal condition, 20, nocturnal condition. The above two materials are stained with 0.001% brilliant cresyl blue. (×480).

Figs. 21, 22. Longitudinal section of petiole parenchyma. 21, chloroplast in direct rays of the sun. 22, chloroplast in nocturnrl condition. The above two materials are stained with 0.001% rhodamin B (×1,150).

plasts. Generally speaking, osmium tetraoxide fixes the threads and tannin vacuole in a black color, while the chloroplasts remain colorless. The results are illustrated in Figs. 24, 25 and 26. Under diurnal conditions, tannin vacuole are fixed in a spherical form, while under nocturnal conditions they appear as crushed globes. The thread-like apparatus has a tendency to as assume thick features in the diurnal period. On the other hand, in the nocturnal period threads appear uniformly in the thin, slender form. When the materials are exposed to the sunlight, small particulated granules appear in the chloroplast, as is shown in Fig. 24. On the contrary, under nocturnal conditions, these granules do not as a rule appear under this fixation (Fig. 26). However, under certain conditions these particulated black granules are also observed in the chloroplast in the nocturnal period.

§ 6. Effect of artificial light upon the thread-like apparatus

As is shown in Fig. 23, a petiole with leaves was placed for 5 to 40 hours in a bottle containing tap water. These plants were then exposed to continuous illumination by an incandescent 60-watt filament lamp hanging 1.5 meters above the petiole. For the microscopic obsevation, the preparations were made as has been described above.

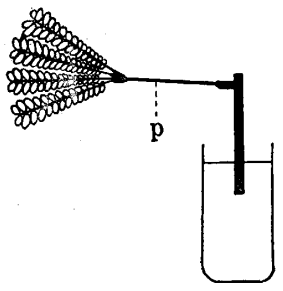
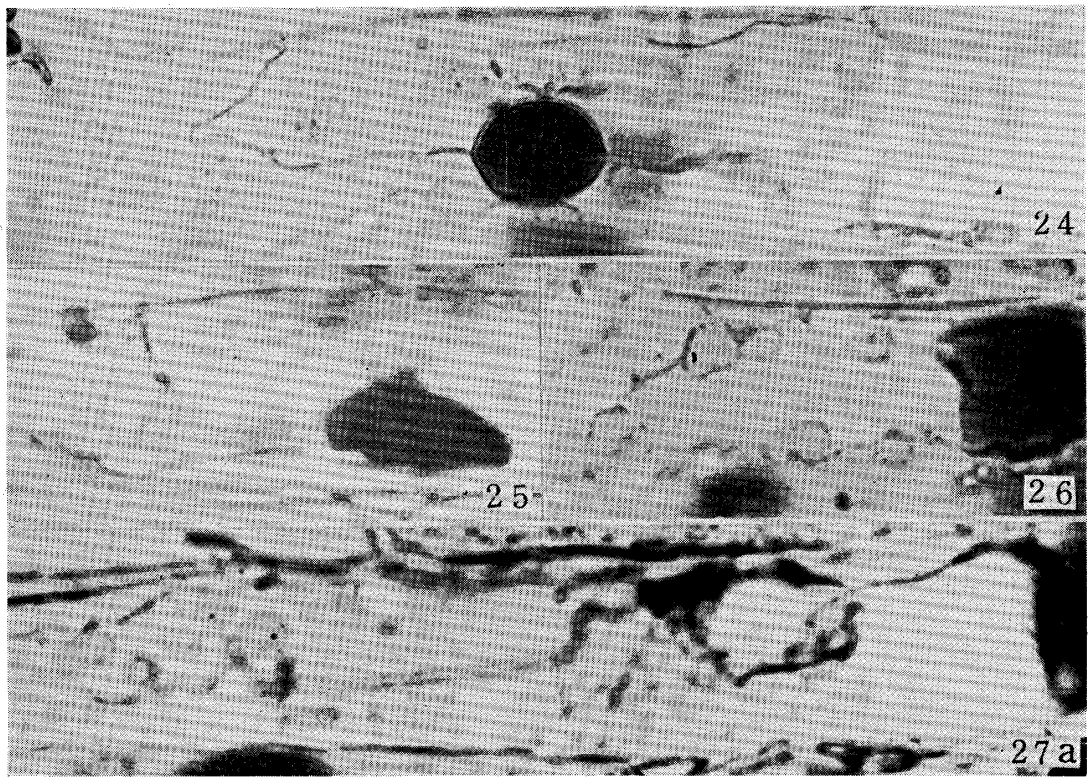
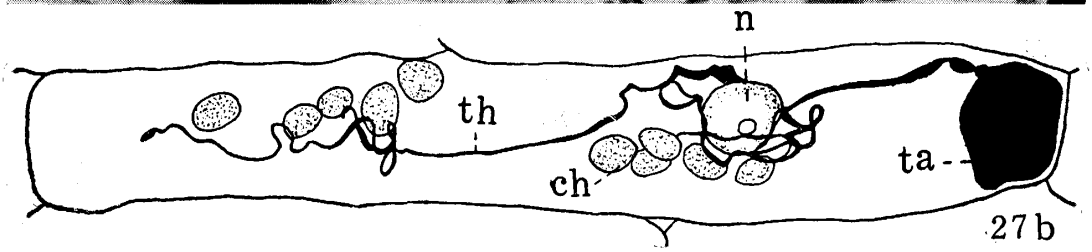


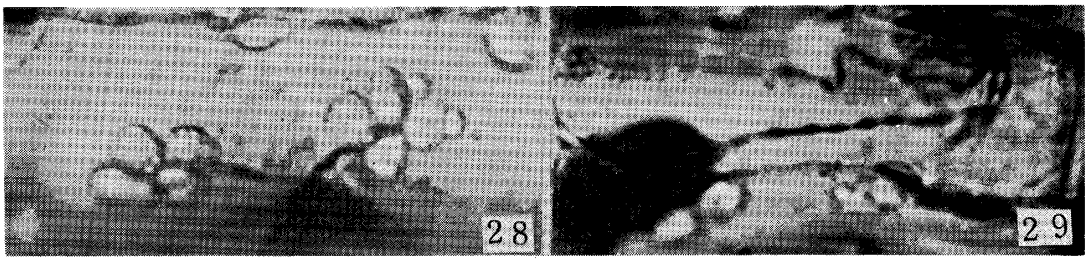
Fig. 23. Apparatus for setting stem with its petiole and leaflets.

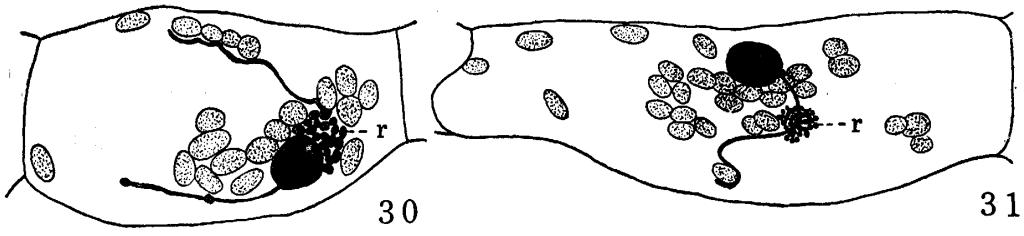
As for the cytophysiological conditions, the material exposed to 10 hours' illumination was the same as the material in the diurnal condition. Upon 25 to 40 hours' illumination, however the thread had a tendency to form a cluster around the nucleus, as is shown in Figs. 27a, b. Furthermore, it was observed that several chloroplasts gathered around a certain part of the thread-like apparatus (Fig. 28). Occasionally, thread appeared to be divided in to the form of a rosary (Fig. 29).

The plants which were cultured in the tap water, as was indicated above, were kept in continuous darkness. During 90 to 120 hours' culture in the darkroom, an interesting phenomenon was observable. The preparations of such material give a clear picture a thread-like apparatus in each cell. This thread develops to such an extent as to assume a long, slender shape, while some part of the thread becomes rather bead-like, as is indicated in Figs. 30 and 31. The plants do not perish, although they are kept in darkness for a very long period. However it seems probable that this phenomenon is an abnormality of the thread-like apparatus.









Figs. 24-31. Longitudinal sections of parenchyma. 24, chloroplast and thread in direct rays of sun. 25 and 26, nocturnal condition. 27a, b, 28 and 29, parenchymatous cell when continuously illuminated for 30 to 40 hrs. 27b, schematic drawing of Fig. 27a. 28, 29, parenchymatous cell continuously illuminated for 25 to 40 hrs. th: thread-like apparatus; ch: chloroplast; n: nucleus; ta: tannin vacuole; r: lamp of bead-like thread.

§ 7. On the function of the thread-like apparatus

By employing cytological methods the author found an interesing phenomenon; *i.e.*, the tannin vacuole and the thread-like apparatus appear differently under diurnal and nocturnal conditions. From this data, it may be concluded that the expansion and contraction of the tannin vacuole in parenchyma occur in the diurnal and in the nocturnal condition respectively. In other words, the quantity of the contents of the tannin vacuole and of the thread-like apparatus tend to increase more in the diurnal period than in the nocturnal period. Such a variation following physiological functional changes experimentally evoked is schematically indicated in Fig. 32a, b. It is probable that the functional significance of the thread-like apparatus accumulation of the tannin substance, because the carbohydrate generally increases during the daytime and decreases during the nighttime.

Fig. 32a, b. Schematic figure of thread-like apparatus and tannin vacuole. a: daytime; b: nighttime. ch: chloroplast; th: thread-like apparatus; ta: tannin vacuole.

When observed by the Champy-Toriyama method, the thread and granules in the chloroplast appear equally black. This positive reaction to the fixative indicate that both cellular elements contain a cestain osmiophil substance. Thus we can observe the granule in chloroplasts by the vital staining method. For these reasons, the present author considers that the substance in chloroplast is probably of the same chemical nature.

Concerning the supposition about the appearence of granules in the chloroplast, there are two posibilities. First, they are products from the chloroplast due to an increase of photosynthesis. Secondly, they are wast substances produced in the chloroplast due to excess photosynthesis. The chemical nature and the distribution of the thread-like apparatus in plants are expected to be studied in detail in the future.

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